

## Introduction

Electrospray Ionization (ESI) is one of the most common ionization methods in mass spectrometry (MS). A characteristic advantage of ESI compared to other ionization methods is the generation of multiple-charged droplets, which contains the analyte. This has also some adverse consequences, since these multiple-charged droplets can pass through the vacuum stages of the mass spectrometer even into the mass analyzer region[1]. The resulting contamination by

droplets can affect the analytical performance. Therefore, it is important to understand the influence of ion source and chromatography parameters on the aspirated droplet ensemble. An ion trap is used to investigate these aspects, as it is particularly well suited to the study of droplets and droplet debris due to its ability to isolate and fragment different mass ranges.

## Methods

- MS: Thermo Fisher LIT LTQ Velos Pro, isolation and fragmentation
- Analyte: Reserpine in ACN/H<sub>2</sub>O (1:1) with 0.1% Formic Acid

## Variation of Activation Time

- **Activation time:** Time between end of ion injection and the mass analysis (ion ejection).
- Comparison of 10 ms, 1000 ms, 5000 ms activation time
- In all spectra reserpine was investigated after the isolation of the mass ranges  $m/z = 1000 \pm 50$ .
- In the first spectrum only signals in the isolated range was detected without any further signals. In spectrum 2 and 3 also a bare reserpine signal appears.
- Spectra show: No analyte signal with 10 ms Activation time. With increasing activation time, the intensity of the analyte also increases. Reserpine was captured in the isolated species and is released from them in the trap.

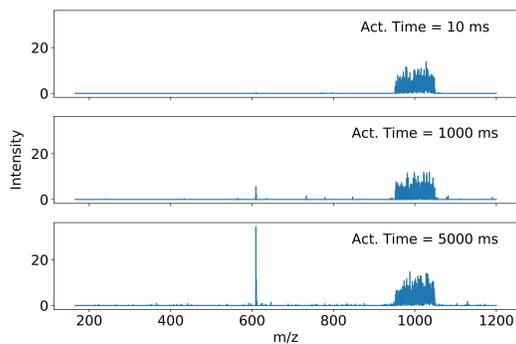


Fig. 1: The mass spectrum of reserpine with three different activation times is shown. In all spectra the mass ranges  $m/z = 1000 \pm 50$  was isolated

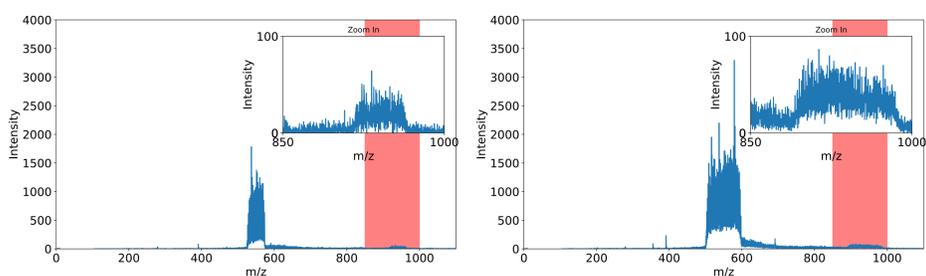


Fig. 2: Left spectrum: Isolation at  $m/z = 550 \pm 25$ . right spectrum: isolation at  $m/z = 550 \pm 50$ . The spectra in the insets show the mass range from  $m/z = 850 - 1000$ . Both spectra have an Activation time of 60000 ms.

- In this measurements the mass range  $m/z = 550 \pm 25$  (left spectrum) and  $m/z = 550 \pm 50$  (right spectrum) was isolated
- Signals in the isolated range are detected
- At higher  $m/z$  value additional signals with lower intensity are observed in both spectra.
- Comparison of both spectra shows that the width of the additional signals are identically to the isolated mass range width.
- This indicates that both signals are directly related. A possible explanation is a  $m/z$  shift due to charge loss, resulting in higher  $m/z$  values.

## Acknowledgement

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## Variation of Helium Pressure

- Helium gas is used to cool down the arriving ions in the ion trap. Additionally, it is used as a collision gas in the trap during the activation and fragmentation process. In this measurement the Helium pressure was turned off.
- Isolated main signal at  $m/z = 700$  is clearly observed. Additionally there is a second, smaller peak around  $m/z = 760$ . This signal structure, a clear peak and a smaller peak next to it, was also observed at  $m/z = 1100$ , but with lower intensity.

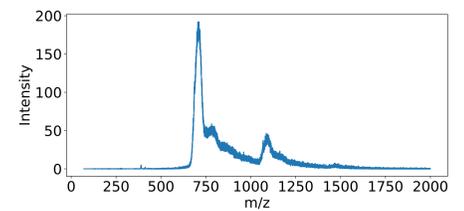


Fig. 3: Measurement of Reserpine with an isolation at  $m/z = 750$  and a width of 0.1. Helium pressure was turn off.

The surprising signal similarity could indicate that both signal groups belongs to the same ion, but with different charge states.

## Variation of Collision Gas

- Helium is used as a collision gas.

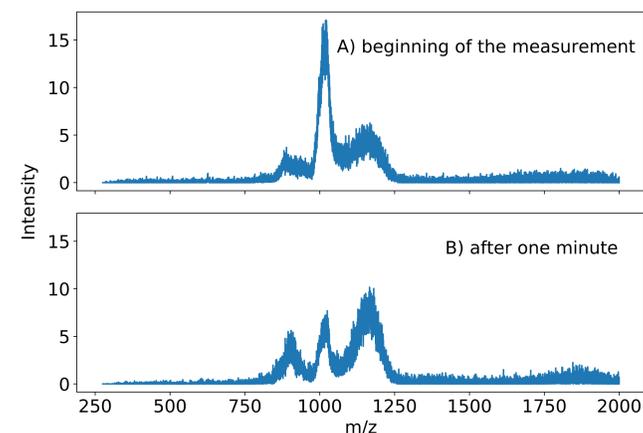


Figure 4: Helium is used as collision gas. The upper panel shows the spectrum just after the gas flow to the MS was opened. The lower panel shows the signals after one minute. In both spectra the signal at  $m/z = 1000 \pm 0.1$  was isolated.

- Two additional peaks at  $m/z = 900$  and  $m/z = 1050$  were observed.
- The intensity of the spectrum is in general lower than previous measurements. The relative signal intensity of the peaks changed during the measurement.

## Summary and Outlook

- The experiments show that charged droplets reach the mass analyzer region of the Linear Ion Trap.
- The experiments show that the intensity of the bare analyte signal depends strongly on the activation time and the used collision gas.
- The analyte signal shows strong dependency on the Helium pressure. Mass and charge loss of the initially isolated species was observed.

## References

- [1] Markert, C.; Thinius, M.; Lehmann, L.; Heintz, C. Stappert, F.; Wissdorf, W.; Kersten, H.; Benter, T.; Schneider, B. B.; Covey, T. R., „Observation of charged droplets from electrospray ionization (ESI) plumes in API mass spectrometers”, Analytical and bioanalytical chemistry, 2021, 413, pp. 5587-5600